FIRE-CURED TOBACCOS

Phenol, Nicotine, and Bacterial Contents

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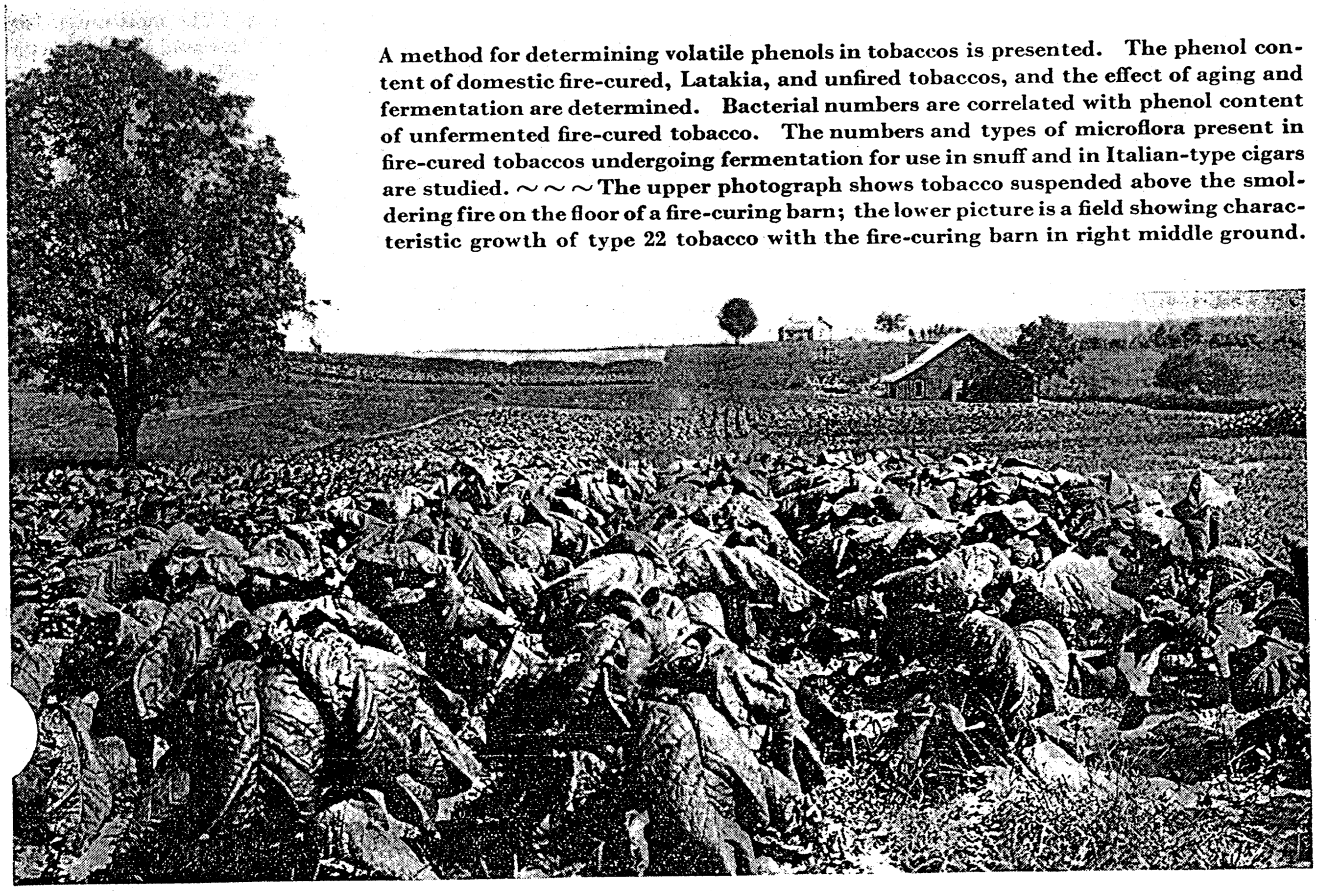
A method for determining volatile phenols in tobaccos is presented. The phenol content of domestic fire-cured, Latakia, and unfired tobaccos, and the effect of aging and fermentation are determined. Bacterial numbers are correlated with phenol content of unfermented fire-cured tobacco. The numbers and types of microflora present in fire-cured tobaccos undergoing fermentation for use in snuff and in Italian-type cigars are studied. ~ ~ ~ The upper photograph shows tobacco suspended above the smoldering fire on the floor of a fire-curing barn; the lower picture is a field showing characteristic growth of type 22 tobacco with the fire-curing barn in right middle ground.



TOBACCO cured in an atmosphere of wood smoke similar to that used for smoking meats and fish is known as fire-cured tobacco, and includes United States types 21, 22, 23, and 24, depending on the origin. In this country tobacco is fire-cured in certain sections of Virginia, Kentucky, and Tennessee. The product is utilized in the manufacture of the Italian type of cigar and for snuff. A similar product is prepared at Latakia in the Levant by curing the small leaves of Turkish types in smoke. The flavor of this product closely resembles that of the American fire-cured types.

When tobacco is fire-cured, the various constituents of the wood smoke are deposited on the leaf and give it the characteristic flavor. Hawley and Wise (*6*) list fourteen phenols, including catechol, that occur in the products of wood distillation; most, if not all, of these may be expected to be present in the leaf of fire-cured tobacco. Little is known about their influence on the chemical changes that take place when the leaf is fermenting.

Tobacco cured without the use of smoke normally contains phenolic constituents, but little is known of their nature. The so-called polyphenols used by Shmuk as indicators of quality in tobacco include, apparently, phenolic acids (*11*) derived from chlorogenic acid and rutin, and possibly other unidentified substances. The presence of catechol, together with small quantities of unidentified phenols, has been reported in tobacco smoke (*7, 8, 9, 12*). In view of the fact that imports of Latakia tobacco in this country had been curtailed as a result of war conditions, it was decided to investigate the possibility of substituting the domestic fire-cured tobacco for the oriental product. Data relating to the phenol and nicotine contents of fire-cured tobaccos have been obtained.

Since the phenols are well-known bacteriostatic and antiseptic agents, it was of interest to determine whether the microfloras of fire-cured tobaccos of different origin differ from those of unfired tobaccos. Further interest was attached to this question in view of one theory that the fermentation of tobacco is a bacterial process and not dependent upon leaf enzymes. If this theory be true, a sterile tobacco should be incapable of autofermentation. Some bacterial counts of fire-cured tobaccos in active fermentation were obtained, but no attempt was made to study the bacterial phenomena accompanying fermentation through all its stages. The work has been temporarily suspended because of war exigencies; but since published data referring to fire-cured tobacco are few, and there is little in print concerning the phenolic content of tobacco, it is thought desirable to publish the data already obtained.

DESCRIPTION OF TOBACCOS

Nineteen samples, representing crops harvested in 1939, 1940, and 1941, were used for this investigation. Thirteen were samples of domestic fire-cured tobaccos, three were samples of Latakia taken from bales, and three were samples of representative nonfired types. Of the unfermented samples of fire-cured tobacco, five were leaf tobacco obtained from tobacco warehouses, one had been cut in preparation for fermenting, and three were Latakia of different degrees of fire curing. Seven samples of fire-cured tobaccos in various stages of fermentation were obtained from cigar and snuff manufacturers. Samples 42-10 to 42-13, inclusive, were selected from lots of tobacco being processed for snuff manufacture; samples 42-14 to 42-16, inclusive, were obtained from lots designed for cigar manufacture. Five of these consisted of unmixed fire-cured tobacco, and two were blends of air-cured (type 35) and fire-cured (type 21) tobaccos. The behavior of the mixtures was not essentially different from that of the unmixed samples.

For comparison, samples of three air-cured types were used, including Pennsylvania cigar leaf (type 41), Maryland cigaret leaf (type 32), and a sample of unfired Kentucky tobacco of a type (22) usually cured by firing. The latter sample (No. 41-6) had been air-cured and served as a comparative material for judging the effects of fire curing on the same type of tobacco. In view of the differences between the fermenting of tobacco snuff and for cigars, it is thought desirable to describe the processes, particularly as there were differences in the microfloras and in the phenol content of the products.

In processing mixed air-cured and fire-cured tobaccos for snuff (samples 42-10 and 42-11), the tobacco, which contains approximately 13% moisture, is removed from the hogsheads in which it has been stored, cut into large pieces, and sprayed with water until it contains 37.5% moisture. It is repacked in hogsheads to undergo the fermentation process, termed the "sweat". During the repacking, additional water is sprayed on the tobacco to maintain the moisture content at 37%. After 7 weeks the process of fermentation is complete. At that time the tobacco usually contains 26.5% moisture.

Eastern dark-fired tobaccos (samples 42-12 and 42-13) are moistened to 39%. The sweat is conducted in a similar manner, and the final moisture content is nearly the same—i. e., 26.5%.

When the tobacco is intended for Italian-type cigars (samples 42-12 to 42-16, inclusive) the fermentation is conducted in bulks termed "formations". After the leaf is moistened and allowed to soften, it is stemmed. The moisture content ranges from 40 to 45%. The stemmed leaf, known as strips, is piled in a regular way in bulks and allowed to ferment until the temperature reaches 104° F., which takes about 5 days. The bulk is then torn down, and the tobacco is cooled slightly, aerated, and moistened if necessary. Then the bulk is rebuilt. After approximately 5 days the temperature reaches 110° F., and the bulk is again dismantled. This process of alternately building up and tearing down the bulks continues for six "formations", each is allowed to reach a higher temperature than the preceding bulk, until the last attains 140° F. It is now ready for the final "conditioning" before being made into cigars.

ANALYTICAL AND BACTERIOLOGICAL DATA

Moisture was determined by heating the sample at 135° for 35 minutes in a Brabender apparatus. The moisture contents of the samples as they were received and used for the bacteriological examinations are presented in Table I. For chemical analysis, the samples were air-dried and ground to fine powder. The moisture contents reported in Table II refer to these dried samples.

Nicotine was determined by the Avens and Pearce method (*2*). Since no satisfactory procedure was available for the determination of total phenols, the method consisted of distilling the phenols with steam from the acidified samples, treating the distillate with the Folin phenol reagent (*4*), determining the light absorption at 7000 Å., and comparing the distillate with a standard after correcting for nonphenolic volatile reducing substances. The results obtained are regarded as reasonably accurate but not precise.

The sample (0.1–1.0 gram), weighed into a 300-cc. Kjeldahl flask, is acidified with 0.5 cc. of 2 *N* sulfuric acid, and 10 cc. of distilled water are added. Steam is passed through the mixture and the distillate is collected in a 250-cc. volumetric flask, the rate being adjusted so that 225 cc. are collected in 20 to 25 minutes. The condenser is rinsed into the receiver and the distillate is made up to 250 cc. A 50-cc. aliquot is placed in a 100-cc. volumetric flask, 5 cc. of the Folin reagent are added and permitted to react for 5 minutes, and 15 cc. of saturated sodium carbonate solution are added. After 5 minutes the product is diluted to 100 cc. with distilled water and allowed to stand at room temperature for 20 minutes. The absorption value at 7000 Å. is then determined. A correction is applied, and the resulting figure compared with that obtained from a standard.

The standard was obtained by treating a series of catechol solutions ranging in concentrations from 0.01 to 0.5 mg. with the

reagents and determining the absorption values at 7000 Å. By plotting log I_0/I against concentration, a nearly linear curve was obtained, which was used for estimating the concentration of phenols in the samples being analyzed. The greatest absorption per unit concentration of catechol was observed in the red end of the spectrum; therefore, the wave length 7000 Å. was chosen as a convenient reference point. The spectral absorption curve in the visible spectrum showed a gradually increasing absorption from 4000 to 7500 Å. with no absorption maxima. The tobacco distillates and resorcinol gave similar curves.

TABLE I. MOISTURE CONTENT OF ACTIVELY FERMENTING FIRE-CURED TOBACCOS AS RECEIVED IN THE LABORATORY

Sample No.	Type	Moisture, %	Stage of Fermentation
42-10	Snuff blend	26.48	4 weeks
42-11	Snuff blend	26.44	7 weeks
42-12	Eastern fired	31.76	Start
42-13	Eastern fired	31.48	25 days
42-14	Eastern fired	39.96	Green formation
42-15	Eastern fired	39.08	5th turning
42-16	Eastern fired	37.25	6th turning

The correction was established by determining the nonphenolic reducing substances that distil at a fairly constant rate and still appear after all the phenols have been carried over. These appear to result from the action of the acid upon various constituents of the tobacco. In our experiments they were equivalent in reacting power to 0.03 mg. of catechol per 50 cc. of distillate. This correction is somewhat arbitrary, and the values reported in Table II for the total phenols, calculated as catechol, are to be regarded as approximations.

The tobaccos were studied according to the technique developed by Reid *et al.* (*5*, *10*) for the determination of actively reproducing bacteria on tobacco. The samples were plated on beef extract–peptone–agar and incubated at 37–38° C. Representative colonies were isolated from countable plates, and the microorganisms classified morphologically.

DISCUSSION OF RESULTS

As was to be expected, the results (Table II) showed that the phenol content of fire-cured tobaccos was greater than that of comparable air-cured tobaccos. The average phenol content of the five unfermented samples of fire-cured tobaccos was 0.316%, whereas the average of the three air-cured tobaccos was 0.021. The unfired sample of type 22 tobacco had 0.016% phenols, or approximately one twentieth of the average for the fire-cured samples. The phenol content of the Latakia samples averaged 0.626%, or twice as much as the average for domestic fire-cured tobaccos. This was expected in view of the heavy tarry coating on the samples.

The average phenol content of the samples taken from actively fermenting lots was 0.017%, or of the same order as unfired tobacco. Similar tobacco samples (42-12, 42-14) of the 1939 eastern fire-cured crop, taken before brisk fermentation began, averaged 0.035%, or about twice as much as the fermented and about one ninth that of the unfermented fire-cured samples. The indications are that the phenols are gradually converted into other substances, probably by oxidation during storage.

The average nicotine content of the actively fermenting lots was 3.34%, somewhat lower than the average for the unfermented samples which was 4.79% or for those taken before brisk fermentation, 4.81%. The average loss amounted to slightly more than 30%, which is in accordance with the usual experience during the fermentation of tobacco in the course of manufacture. The nicotine content of the Latakia samples was very low. However, Turkish tobaccos are known to contain smaller quantities of this alkaloid than domestic types (*1*). No evidence was obtained to indicate any correlation between the nicotine content and the number of bacteria per gram in these samples. Apparently the microflora is unaffected by wide differences in the nicotine content of the substrate.

The results of the bacterial study show a definite correlation between the number of microorganisms and the degree of firing. The fact that only spore formers and micrococci were found suggests that only the more resistant forms were able to survive the treatment, and even those survived only in small numbers. Sample 42-18, which showed Gram-negative rods, was an exception. Since it was a ground sample, they may have represented a contamination.

The sample of unfired type 22 (No. 41-6) contained practically a pure culture of *Bacillus subtilis*. Since this was tobacco of the 1940 crop, it suggests that multiplication had occurred, possibly during a sweat. It is not uncommon for tobacco leaf to contain large numbers of organisms, as demonstrated by other workers (*3*, *5*, *10*).

The samples of fermented tobaccos were obtained when the bulks were being remade or initially laid down. They were carefully packed in sealed cans and rushed to the laboratory, where they were immediately placed in a refrigerator at 40° F. to keep changes at a minimum until the material could be cultured. Seven days elapsed from the taking of the sample to culturing. This fact should be given consideration when the data obtained from the fermented samples are interpreted. It may explain the high count on sample 42-12. Also it should be pointed out that, as the samples of fermented tobaccos do not represent progressive stages of a single lot of tobacco but are from different lots of similar tobaccos, the changes in types and numbers of bacterial flora between the various stages are not to be considered absolute but merely suggest trends.

TABLE II. NICOTINE, PHENOL, AND BACTERIAL CONTENTS OF FIRE-CURED AND AIR-CURED TOBACCOS

Sample No.	Type of Tobacco	U. S. Type	Crop Year	Stage of Fermentation	Moisture, %	Nicotine, %[a]	Phenols as Catechol, %[a]	No. of Bacteria per Gram			
								Total	Spore formers	Micrococci	Others
41-3	Pa. cigar leaf	41	1940	Unfermented	11.40	4.95	0.038	43,000	43,000	...	...
41-6	Unfired	22	1940	Unfermented	7.45	2.92	0.016	3,200,000	3,200,000	...	...[b]
41-8	Md. cigaret leaf	32	1940	Unfermented	8.95	1.25	0.010	61,000	26,200	8,600	26,200
41-15	Eastern fired	22	1940	.....	11.72	5.63	0.11	100	100	...	...
42-2	Latakia, abouriha	90	..	.....	11.96	0.55	0.62	200	200	...	...
42-3	Latakia, Bayr	90	..	.....	10.36	0.26	0.34	<100	...	...	...
42-4	Latakia, av.[c]	90	..	.....	11.16	0.24	0.92	<100	...	...	...
42-5	Western fired	23	1941	Unfermented	8.10	4.23	0.41	25,000	25,000	...	...
42-6	Eastern fired	22	1941	Unfermented	7.75	5.27	0.17	4,000	4,000	...	...
42-7	Eastern fired	22	1941	Unfermented	7.37	3.82	0.26	3,700	2,200	1,480	...
42-8	Eastern fired	22	1941	Unfermented	10.78	4.69	0.32	1,000	750	250	...[b]
42-18	Eastern fired	22	1941	Unfermented	7.95	5.95	0.42	1,200	300	600	300[e]
42-10	Snuff blend[d]	..	1939	4 weeks	6.07	2.29	0.013	2,200,000	...	440,000	1,760,000[e]
42-11	Snuff blend[d]	..	1939	7 weeks	6.07	2.42	0.013	740,000	...	...	740,000
42-12	Eastern fired	22	1939	Start	6.13	3.76	0.034	5,000,000	...	5,000,000	...
42-13	Eastern fired	22	1939	3 weeks 4 days	8.28	3.84	0.017	1,900,000	...	380,000	1,520,000[e]
42-14	Eastern fired	22	1939	Start	8.43	5.87	0.036	11,000	2,200	4,400	4,400[b]
42-15	Eastern fired	22	1939	5th turning	9.42	3.94	0.022	106,000	...	106,000	...
42-16	Eastern fired	22	1939	6th or last turning	9.48	4.24	0.022	22,000	4,400	17,600	...

[a] Moisture-free basis. [b] Gram-negative rods. [c] District of production unknown. [d] Mixture of Virginia fire-cured and air-cured, types 21 and 35. [e] Yeast-like forms.

In tobaccos fermented for snuff production, the original flora was rapidly supplanted by micrococci. As fermentation progressed, the latter declined in number and were supplanted by numerous yeastlike forms. The latter existed in practically pure culture at the end of the processing of the snuff blend (sample 42-11) and comprised more than three fourths of the flora after 3 to 4 weeks (42-10 and 42-13). From the limited observations, it would appear that the maximum increase in microorganisms occurs during the first week of fermentation and gradually declines as the first floral types are replaced by the other forms.

In the fermentation of dark-fired tobacco (Type 22) for production of Italian-type cigars, yeastlike forms were found in only one sample (42-14). The original mixed flora was supplanted by micrococci, which persisted throughout. The presence of the *B. cereus–B. megatherium* group at the end of fermentation (42-16) probably indicates that they persisted as a minority and were detected only as the count decreased. These results indicate that microorganisms multiply on fire-cured tobaccos under the proper conditions.

The apparent contradiction in the bacterial count of the series comprising samples 42-14, 42-15, and 42-16, in which there is an anomalous increase in the fifth turning followed by a considerable decrease in the sixth turning, is explained by the fact that these are from different lots of tobacco. This fact is evidenced by the nicotine content, which is lower in 42-15 than in 42-16; if the two turnings had originated from the same lot of cured tobacco, the relative nicotine contents would have been reversed.

CONCLUSIONS

The average content of volatile phenols in the unfermented fire-cured tobaccos was fifteen times as great as that of the air-cured tobaccos. The volatile phenol content of fire-cured tobacco diminished during fermentation and aging. Latakia tobacco contained more volatile phenols and less nicotine than domestic samples.

In the unfermented samples the number of bacteria per gram was correlated with the content of volatile phenols. This relation did not hold without exception in actively fermenting tobacco, where the conditions were more complicated. There appears to be no relation between the nicotine content and the bacterial count of the tobaccos studied. Micrococci were the predominating organisms on samples of fire-cured tobacco at the height of fermentation. In samples of tobacco fermented for snuff the micrococci increased initially but were supplanted by yeastlike forms as fermentation progressed. In samples of tobacco fermented for use in Italian-type cigars, the micrococci gained ascendency and persisted to the end. A few spore formers were detected at completion of fermentation when the total count became relatively low.

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